

Hemodynamic effects of the early and long-term administration of propranolol in rats with intrahepatic portal hypertension

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Abstract *Background and aims* The aims of this study were to evaluate a preventive effect on collateral venous circulation of long-term administration of propranolol in intrahepatic portal hypertensive rats. *Methods* Eighty-six Sprague–Dawley rats were allocated to two models of hepatic fibrosis, bile duct-ligated (BDL) induced and carbon tetrachloride (CCl₄) induced. Each model was divided into two groups: one receiving placebo and the other propranolol (75 mg kg⁻¹ d⁻¹). Mean arterial pressure (MAP), heart rate (HR), portal pressure (PP), cardiac index (CI), vascular systemic resistance, and splenorenal shunt blood flow (SRS-BF) were measured in anesthetized rats. *Results*

In the BDL model, no significant hemodynamic changes were observed in the propranolol group compared with the placebo group. In CCl₄-induced rats, HR (390 ± 50 vs. 329 ± 51 beats/min, *P* = .001), CI (44 ± 11 vs. 34 ± 10 ml/min, *P* = .004), PP (15.4 ± 3.0 vs. 13.4 ± 1.9 mmHg, *P* = .045), and SRS-BF (1.4 ± 1.1 vs. 1.0 ± 1.0 ml/min, *P* = .047) were significantly lower in the propranolol group. *Conclusions* This study showed that propranolol has a significant hemodynamic effect only in the CCl₄ model and suggested a model-dependent effect of propranolol.

Keywords Bile duct ligation · Carbon tetrachloride · Collateral venous circulation · Propranolol · Splenorenal shunt

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Introduction

Portal hypertension (PHT) is characterized by the development of portosystemic shunts (PSS). It is well known that collateral circulation contributes to the hyperkinetic syndrome, that is, hemodynamic changes associated with PHT (increase of cardiac output and splanchnic blood flow and decrease of systemic arterial pressure). The development of collateral circulation follows the order: development of hepatic fibrosis, increase of splanchnic blood flow, and plasmatic concentration of vasodilator agent (i.e., nitric oxide) [1, 2]. In cirrhotic patients, esophageal varices more frequently indicate a clinically significant PSS. Variceal bleeding is a medical emergency leading to a higher mortality, which, in spite of recent therapeutic progress, remains a major complication. Propranolol is the main treatment modality used in the primary and secondary prevention of variceal bleeding. The concept of preprimary prevention of variceal bleeding, that is, the

prevention of occurrence of esophageal varices, is debated [3–5]. Two trials performed in cirrhotic patients did not suggest the efficacy of propranolol [3] or timolol [4] for preprimary prevention, whereas another study showed a positive effect of propranolol [5].

In experimental models of PHT, especially with extrahepatic PHT, several studies suggested an effective pharmacologic prevention of PSS development with propranolol [1, 6–8]. Thus, early and long-term administration of propranolol reduces the degree of PHT and the development of PSS in extrahepatic PHT models with portal vein stenosis [3, 6, 8, 9] or prehepatic schistosomiasis [10]. In intrahepatic PHT models, CCl₄ or thioacetamide administration—the long-term administration of propranolol, hinders the development of PSS [11] and significantly decreases portal pressure (PP) [11, 12]. However, in bile duct-ligated (BDL) models, chronic administration of propranolol had no significant effects on PP and PSS [13, 14]. Therefore, these data suggested that hemodynamic effects of propranolol in animals could be model dependent.

In cirrhotic patients, long-term pharmacologic treatment with propranolol is associated with a marked reduction in the long-term risk of developing complications of PHT, independent of the bleeding risk (ascites, spontaneous bacterial peritonitis, hepatic encephalopathy, or hepatorenal syndrome), and with improved survival [15].

The aims of this study were to evaluate the hemodynamic effects, especially regarding collateral circulation of the early and long-term administration of propranolol in two intrahepatic PHT models.

Methods

Animals

Eighty-six male Sprague–Dawley rats (Pet University, Angers, France) were studied. All rats were allocated into two groups according to the intrahepatic PHT model: BDL induced and CCl₄ induced. Each model group was allocated to receive placebo (BDL model, $n = 23$; CCl₄ model, $n = 22$) or propranolol treatment (BDL model, $n = 21$; CCl₄ model, $n = 20$). These models have been described previously [16, 17].

Briefly, regarding the BDL model, the common bile duct was isolated by a double ligature with section between the two ligatures in isoflurane-anesthetized rats. The first ligature was made below the junction of the hepatic ducts. The second one was made above the entrance of pancreatic ducts. The portion of bile duct between the two ligatures was resected to avoid repermeabilization.

In CCl₄ models, cirrhosis was induced by intragastric administration of CCl₄ according to previously described methods [16]. The aim of this “princeps study” was the creation of toxic cirrhosis model in 12–15 weeks with the lowest mortality. However, several studies have shown that the PHT is significantly developed before 12–15 weeks [18, 19]. In this study, we have chosen 8–9 weeks for the CCl₄ model for evaluating PHT because PHT is permanent and stable during this time, and thereby hemodynamic measurements were made with an acceptable mortality. In order to increase the yield of cirrhosis, rats also received phenobarbital in the drinking water. Animals were gavaged weekly and weighed twice a week to assess their response to CCl₄. Forty-eight hours after each dose of CCl₄, weight loss was used to estimate the subsequent doses. In this model, measurements were performed 1 week after discontinuation of phenobarbitone and CCl₄.

In conformity with European legislation for research involving animals, the French Agriculture Office approved protocols performed in our laboratory.

Therapeutic regimen

Propranolol was administered by gavage ($75 \text{ mg kg}^{-1} \text{ d}^{-1}$) for 4 weeks in the BDL model as soon as bile duct ligation was performed and for 9 weeks in the CCl₄ model after the first CCl₄ injection. In both models, the control group was given the vehicle by gavage (1 ml of saline solution). The treatment was started before the apparition of the PHT, just on the modeling day, as is recommended in the previously reported study [8]. This therapeutic regimen underlines the “early” stage of the treatment.

Measurements and observers

Hemodynamics, histopathologic analyses, and biochemistry were performed at 28 days in the BDL model and at 63 days in the CCl₄ model. All rats had free access to food and water until 14–16 h before the study.

Throughout the period of measurements, body temperature was maintained at 37°C for all surgical procedures and measurements with a homeothermic blanket system (Harvard Apparatus Ltd., Kent, England).

Hemodynamics, morphometric fibrosis measurements, and biochemistry were performed by different observers. No observer was aware of the treatment given and the results of other observers.

Hemodynamic measurements

For the experiments, animals were anaesthetized by intraperitoneal injection of thiopental at a dosage of 50 mg kg^{-1} body weight. Mean arterial pressure (MAP) and heart rate

(HR) were measured with pressure transducer (Mallinckrodt, Medica, Dublin, Ireland) connected to the left femoral arterial catheter. The abdomen was opened and a polyethylene catheter (0.7-mm diameter) was inserted into a small ileal vein and gently advanced up to the bifurcation of the superior mesenteric and splenic veins to measure PP with pressure transducer. Pressures and HR were monitored using a multichannel recorder (Nikon Kohden, Tokyo, Japan).

In PHT rats, the main PSS is the splenorenal shunt (SRS), while the collateral esophageal venous network is poorly developed [20]. Measurement of SRS blood flow (SRS-BF) was performed by using a transit-time ultrasound (TTU) technique, which consists of a volumetric blood measurement flow using a perivascular flow probe. SRS was carefully dissected, and any trace of fatty tissue was removed from the vessel to prevent obstruction of the ultrasound signal. A 1-mm TTU flow probe (1RB, J reflector, 1-mm diameter, Transonic Systems Inc., Ithaca, NY) was inserted in the isolated section of this vessel near its junction to the left renal vein. Blood flow was measured on a flowmeter for small animals (T 206 XM dual channel, Transonic Systems Inc., Ithaca, NY) [21].

Cardiac output was measured using TTU-dilution method as previously described using the same flowmeter as used for SRS-BF measurements (T 206 XM dual channel) [22]. Briefly, the ultrasound blood velocity varies from 1,560 to 1,590 m s^{-1} and is known to be mainly dependent on protein concentrations and blood temperature. The ultrasound velocity in saline solution at 37°C is 1,533 m s^{-1} . Injection of a saline bolus (NaCl 0.9%) into the venous bloodstream at this temperature generates a transient decrease in ultrasound velocity in arterial bloodstream because of the transient dilution of global blood proteins. Experimentally, a 0.2-ml saline bolus was injected through a femoral vein catheter and the ultrasound velocity dilution curve was recorded with a TTU probe (1RB, J reflector, 1-mm diameter), which was placed around a carotid artery. The dilution curve described an area under the curve (AUC), which was calculated offline using Matlab® 4.2 c software (MathWorks, Natick, MA). Cardiac output was calculated from the following formula: cardiac output = $V/(\text{AUC} \times K)$, where V is the volume of saline injection (0.2 ml) and K the probe constant. K was determined as described previously. Briefly, the TTU probe to be calibrated was placed around the superior mesenteric vein. A 0.05-ml saline bolus (NaCl 0.9%) at body temperature was injected through an ileal vein catheter. This generated an ultrasound velocity dilution curve, and subsequently an AUC_k , recorded from the TTU probe. K was then calculated using the following formula: $K = V_k/(Q \times \text{AUC}_k)$, where V_k is the volume of saline injection (0.05 ml); Q is the blood flow (ml min^{-1}) detected by the TTU probe in the superior mesenteric vein, and AUC_k is

the area under the ultrasound velocity curve ($V \text{ min}$). Cardiac index (CI) was then calculated using the following formula: $\text{CI} = \text{Cardiac output}/(\text{Body weight}/100 \text{ g})$.

Hemodynamic measurements were performed 30 min after manipulations, that is, when values were stabilized.

Serum function tests

At the end of hemodynamic measurements, blood samples (from femoral artery) were immediately centrifuged at 4°C and serum was kept at −20°C until the assays were performed. Blood liver and renal function tests including total bilirubin, alkaline phosphatases (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities, and creatininemia were performed on each rat.

Statistics

Quantitative variables were expressed as mean \pm SD. The statistical differences between the groups were compared using a one-way analysis of variance with the Bonferroni multiple comparison tests as required and unpaired t -tests. Statistical significance was assumed if $P < .05$. Correlations were calculated using Pearson coefficients. Statistical evaluations were performed using SPSS software, Version 11.5.1 (SPSS Inc., Chicago, IL).

Results

General characteristics of rats

In the CCl_4 model, initial body weight was significantly lower in propranolol-treated rats than in placebo rats. In each model, final body weight of treated rats was significantly lower than that in the placebo group. However, for each model, the weight gain, expressed in percentage, was not significantly different between placebo and treated rats (Table 1).

Final body weight, gain body weight, and liver body weight showed a significant difference between the two placebo groups, with gain body weight lower and a final liver weight higher in the BDL model than in the CCL_4 model. These results suggested that the BDL model was more aggressive than the CCL_4 model.

Hemodynamic studies

In placebo groups, MAP, systemic vascular resistance (SVR), and CI were significantly different between the BDL and CCL_4 models. MAP and SVR were higher and CI was lower in the CCl_4 model than in the BDL model. Moreover, PP appeared higher in BDL than in CCl_4 rats. In the BDL model, no significant hemodynamic change was observed in

Table 1 General characteristics of BDL and CCl₄ rats

	BDL			CCl ₄		
	Placebo (n = 23)	Propranolol (n = 21)	P-value	Placebo (n = 22)	Propranolol (n = 20)	P-value
Initial body weight (g)	250 ± 47	242 ± 26	0.51	261 ± 29	238 ± 23	0.01
Final body weight (g)	337 ± 28	313 ± 44	0.02	405 ± 41 ^a	377 ± 45	0.04
Gain in body weight (%)	39 ± 26	29 ± 14	0.31	57 ± 21 ^a	60 ± 24	0.51
Liver weight (g)	22 ± 4	22 ± 5	0.70	19 ± 3 ^a	17 ± 5	0.20

^a Significantly difference with placebo rats

Table 2 Systemic and splanchnic hemodynamics of BDL and CCl₄ rats

	BDL			CCl ₄		
	Placebo (n = 23)	Propranolol (n = 21)	P-value	Placebo (n = 22)	Propranolol (n = 20)	P-value
Mean arterial pressure (mmHg)	103 ± 12	105 ± 15	0.630	117 ± 19 ^a	112 ± 23	0.428
Heart rate (beat min ⁻¹)	382 ± 56	353 ± 64	0.151	390 ± 50	329 ± 51	0.001
Cardiac index (ml min ⁻¹)	59 ± 17	50 ± 19	0.088	44 ± 11 ^a	34 ± 10	0.004
Systemic vascular resistance (dyn s cm ⁻⁵ 100 g ⁻¹ 10 ³)	152 ± 47	198 ± 88	0.148	227 ± 64 ^a	287 ± 98	0.056
Portal pressure (mmHg)	18 ± 3	17 ± 3	0.851	15 ± 3	13 ± 2	0.045
Spleno-renal shunt blood flow (ml min ⁻¹)	2.2 ± 2.5	1.4 ± 1.5	0.201	1.4 ± 1.1	1.0 ± 1.0	0.047

^a Significantly difference with placebo rats

propranolol-treated rats. There was only a tendency for decrease in CI suggesting minimal effective propranolol effect (Table 2).

Compared with the placebo groups, propranolol administration in CCl₄ rat induced a significant decrease in HR, CI, PP, and SRS-BF, but no significant changes in MAP and SVR.

In the BDL and CCl₄ models, there was no significant correlation between PP and SRS-BF, neither in placebo rats ($r = 0.14$, $P = .53$; $r = -0.47$, $P = 0.83$, respectively) nor in propranolol-treated rats ($r = -0.11$, $P = 0.63$; $r = -0.37$, $P = 0.1$, respectively).

Biochemical tests

In the BDL and CCl₄ models, propranolol administration did not significantly change the biochemical parameters (Table 3).

Discussion

To our knowledge, this is the first study to evaluate the effects of early and long-term administration of propranolol on collateral blood flow with a direct method of volumetric measurement of blood flow (TTU) in intrahepatic PHT

models in rats. This subject is especially clinically relevant in the context of preprimary prevention of variceal bleeding [3] (Table 4).

Hemodynamics

Our study showed significant different hemodynamic effects of long-term administration of propranolol between intrahepatic PHT models based on BDL and CCl₄.

We found that long-term administration of propranolol in the CCl₄ model had significantly beneficial splanchnic hemodynamic effects, preventing the increase of PP (−13%) and of SRS-BF (−36%). Effective β -blockade was suggested by a significant decrease in HR and CI associated with an increase in SVR. These results are in agreement with the results reported in extrahepatic PHT (PVL and chronic schistosomiasis), showing reductions of respectively −23 and −28% for PP and −43 and −80% for PSS as assessed by a microsphere method [8, 10].

In our study, we pointed that the hemodynamic variations on collateral circulation were assessed with a TTU technique, a direct method of volumetric blood measurement flow. The SRS is the main collecting vessel of the

Table 3 Blood biochemical parameters of BDL and CCL₄ rats

	BDL			CCL ₄		
	Placebo (n = 23)	Propranolol (n = 20)	P-value	Placebo (n = 22)	Propranolol (n = 18)	P-value
Urea (mmol/l)	9.6 ± 2.5	8.3 ± 1.7	0.1	8.3 ± 5.5 ^a	7.1 ± 1.1	0.7
Creatinine (±mol/l)	68 ± 19	58 ± 8	0.1	63 ± 13	62 ± 11	0.8
Aspartate aminotransferase (UI/l)	583 ± 443	779 ± 445	0.06	229 ± 95 ^a	226 ± 100	0.9
Alanine aminotransferase (UI/l)	74 ± 36	83 ± 37	0.4	83 ± 34	73 ± 26	0.6
Alkaline phosphatases (UI/l)	323 ± 91	283 ± 50	0.2	159 ± 75 ^a	166 ± 56	0.4
Bilirubin (μmol/l)	131 ± 29	114 ± 19	0.07	3.6 ± 2.1 ^a	5.3 ± 2.2	0.01

^a Significantly difference with placebo rats

Table 4 Percent of rat developed ascites in each model

	BDL model			CCL ₄ model		
	Placebo (n = 23)	Propranolol (n = 21)	Placebo +propranolol (n = 44)	Placebo (n = 22)	Propranolol (n = 20)	Placebo +propranolol (n = 42)
% of rat with ascites	52	47	50	40	65	52

collateral circulation in portal hypertensive rats [23]. The determination of SRS-BF by TTU method was shown as an accurate index of collateral circulation, which was well correlated with the quantification of PSS by a microspheres method [20]. A TTU method appears more suitable than a microsphere method. This latter method expresses PSS as a fraction of splanchnic blood flow shunt (%PSS). Indeed, if portal tributary blood flow (PT-BF) and PSS blood flow vary in the same proportion, %PSS will remain paradoxically unchanged because %PSS = PSS blood flow/PT-BF [21].

Classically, propranolol administration results in a decrease of portal tributary blood flow as well as a decrease of cardiac output, despite increase of portal tributary vascular resistance [24]. It has been postulated that the increase of portal tributary vascular resistance is due to an unopposed α -adrenergic activity or to the β_2 -decreasing property of propranolol [8, 25].

Thus, the effect of propranolol on collateral circulation can be due to a decrease in PP and a direct vasoconstriction. In our study, no significant correlation existed between PP and SRS-BF in the propranolol group. This lack of correlation after treatment suggests a direct effect on SRS with an increase in vascular resistance. These findings were similar in cirrhotic patients [26], and suggested that propranolol might have a direct effect on SRS by acting through intermediate β -receptors [25, 27]. This direct vascular effect on

SRS might provide the explanation that the hemodynamic effect of propranolol would be relatively more marked on SRS-BF than on PP. Moreover, an increase in vascular resistance of SRS could offset the benefit of propranolol in terms of PP reduction. We have observed this apparent discrepancy with several drugs evaluated in the same conditions: chronic octreotide treatment in CCL₄ and BDL rats [28]. These results may explain that, despite small decreases in PP measured by portal sus-hepatic gradient, propranolol is effective in preventing the incidence of variceal bleeding in cirrhotic patients, mainly by reducing collateral circulation blood flow, as shown by azygos blood flow measurements [29]. Conceptually, a reduction in portal venous pressure should result in improvement at PSS level with decrease of PSS development. This hypothesis was supported by observing extrahepatic [3, 6, 8, 9] or prehepatic models [10]. Similarly, a decrease of collateral blood flow consecutive to an increase of collateral vascular resistance shunt should result in an opposite effect to the development of collateral shunting.

In our study, the value of SRS-BF in treated rats suggested a preventive effect of propranolol on collateral circulation in the CCL₄ model. The preventive effect on the development of the collateral venous circulation predominates the action of vasomotor effect, because the low outflow values of SRS-BF, despite the development of hepatic fibrosis, strongly suggest an effect on the development of collateral circulation

for two reasons: First, such dramatic hemodynamic data have never been observed in acute or chronic delayed administration, that is, when fibrosis or PHT was already acquired. Second, these values of SRS-BF are similar to that observed in baseline conditions in normal rats [20, 21]. Moreover, the macroscopic aspect of SRS in treated rats was similar to normal rats. The development of varices does not depend on hemodynamic changes on PP alone.

In BDL rats, the long-term administration of propranolol did not significantly decrease PP or SRS-BF. These results are in agreement with previous studies, showing that long-term administration of propranolol did not significantly change splanchnic hemodynamics in this intrahepatic PHT model of rats and dogs [13, 14].

How can we explain the absence of the effect of propranolol in the BDL model?

- A dose-dependent effect (ineffective β -blockade dose) of propranolol is not likely. Indeed, the dose used was similar to that used in other studies with significant effects [8, 10]. A pitfall of our study was the lack of plasmatic dosage of propranolol. However, we observed that patent changes in CI to around 15% in the BDL model did not significantly differ with the changes observed in the CCl₄ model (–22%).
- One of the drawbacks of the BDL model is the potential formation of a biliary cyst, which may compress the portal vein and disturb the measurement. In our study, a very low incidence of the formation of biliary cyst was noted (data not registered). However, in our study, the existence of biliary cyst has not influenced the hemodynamic measurements.
- The CCl₄ model had a longer duration of propranolol administration than the BDL model. However, in our study, we have used 2 different models of PHT. We observed the hemodynamic effect of propranolol for each model when the PHT was developed, stable, and permanent. We have not directly compared the hemodynamic parameters of the 2 models. Furthermore, after 9 weeks in the BDL model, the rats were too ill to undergo hemodynamic measurements and mortality was very high.
- The BDL model could appear less sensitive to the effects of the propranolol in comparison with the other PHT models, especially extrahepatic PHT models:
 - i. The BDL model is characterized by a sudden, high, rough, and marked PHT. Propranolol effects are blunted when PP is increased or when collateral circulation is more developed [30].
 - ii. The BDL model is characterized by a more severe hepatic disease than other PHT models. Mean values of PP and SRS-BF were higher in BDL rats, suggesting a more severe PHT in the latter model.

The level of PP in BDL rats is higher than that in CCl₄ rats and has already been reported [21]. The gain in body weight appears 2 times lower in the BDL model than in the CCl₄ model, underlying a higher level of severity of PHT in this model. Indeed, this model is due to an acute and definitive bile duct ligation, with sudden and high increase of PP. In the CCl₄ model, the development of PHT is more progressive. In the BDL model, PHT is rapidly associated with manifestations of liver failure (jaundice) as well as with a more important development of venous collateral circulation than in the CCl₄ model. Propranolol does not seem to influence the gain in body weight because in both models, it did not significantly differ between treated and placebo rats. The relation between severity of PHT and effects of propranolol was already suggested in cirrhotic patients. Thus, effects of propranolol were more marked in patients with Child-Pugh A or B than in patients with Child Pugh C [30].

It has been reported that β -adrenergic receptor function is impaired in BDL rats, with a cardiac hyporesponsiveness to β -stimulation [31]. At the cardiac level, BDL rats show a significant downregulation of β -adrenoreceptor density compared with sham-operated control or portal vein stenosis rats [32].

- Cholestasis, as well as increase of biliary acids, could blunt propranolol effect. Indeed, biliary acids might produce splanchnic vasodilation and mask the propranolol effects [33].
- The BDL model is characterized by intense bacterial translocation; a higher incidence of bacterial translocation in cirrhotic patients with obstructive jaundice has been reported [34]. *Bacterial translocation*, defined as the migration of bacteria from the intestinal lumen to mesenteric lymph nodes or other extra-intestinal sites, has been suggested to be involved in the pathogenesis of severe infections in cirrhosis [35]. The associated proinflammatory cytokine response exacerbates hepatic dysfunction, encephalopathy, and hemodynamic disturbance that underlie the development of PHT, hepatorenal syndrome, or septic chock [36]. A bacterial translocation was reported in the CCl₄ and BDL models [36]. In the BDL model, bacterial translocation seemed to be more marked than in the CCl₄ model because of higher overgrowth of bacteria secondary to PHT level, as well as to biliary acid reduction in gastrointestinal tract [37]. Recent studies have suggested a significant effect of propranolol on survival, independent of variceal bleeding events [38]. This beneficial effect could result in part from a improvement of intestinal motility by propranolol, resulting in decrease of bacterial overgrowth [39].

In conclusion, long-term administration of propranolol is model dependent. This may be explained by PHT severity and hepatic disease in the BDL model. Thus, the BDL model might to be used with caution for the evaluation of drugs hemodynamic effects.

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